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APPLICATION NUMBER: *60/422,986*
FILING DATE: *November 01, 2002*
RELATED PCT APPLICATION NUMBER: *PCT/US03/34777*

By Authority of the
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PTO/SB/16 (10-01) Approved for use through 10/31/2002. OMB 0651-0032

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.	ET899942477US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Ira B.	Black	Skillman, New Jersey

☐ *Additional Inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Genes Associated with Memory

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ Customer Number 33348 → *Place Customer Number Bar Code Label here*

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☐ Firm or Individual Name	Margaret Mary Kozik Richardson				
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ENCLOSED APPLICATION PARTS (check all that apply)

☑ Specification *Number of Pages* 23

☑ Drawing(s) *Number of Sheets* 3

☐ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number

☐ Other (specify) Claims (2 pgs); Sequences (3 pgs); Post Card

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☑ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 500756

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($): $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☑ Yes, the name of the U.S. Government agency and the Government contract number are: Unknown at this time.

Respectfully submitted,

SIGNATURE _____ Date 11/01/2002

TYPED or PRINTED NAME Margaret Mary Kozik Richardson

REGISTRATION NO. *(if appropriate)* 47,023

TELEPHONE 732-235-9350

Docket Number: RWJ-02-79

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

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TOTAL AMOUNT OF PAYMENT	($)	80.00

Complete if Known	
Application Number	
Filing Date	Herewith
First Named Inventor	BLACK, Ira B.
Examiner Name	
Group Art Unit	
Attorney Docket No.	RWJ-02-79

METHOD OF PAYMENT

1. ☑ The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:

Deposit Account Number: 500756

Deposit Account Name: University of Medicine and Dentistry of New Jersey

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☑ Applicant claims small entity status See 37 CFR 1.27

2. ☐ **Payment Enclosed:**

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	740	201	370	Utility filing fee	
106	330	206	165	Design filing fee	
107	510	207	255	Plant filing fee	
108	740	208	370	Reissue filing fee	
114	160	214	80	Provisional filing fee	80.00

SUBTOTAL (1) ($)

2. EXTRA CLAIM FEES

		Extra Claims		Fee from below		Fee Paid
Total Claims		-20** =		X	=	
Independent Claims		- 3** =		X	=	
Multiple Dependent					=	

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	84	202	42	Independent claims in excess of 3
104	280	204	140	Multiple dependent claim, if not paid
109	84	209	42	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	920	217	460	Extension for reply within third month	
118	1,440	218	720	Extension for reply within fourth month	
128	1,960	228	980	Extension for reply within fifth month	
119	320	219	160	Notice of Appeal	
120	320	220	160	Filing a brief in support of an appeal	
121	280	221	140	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,280	241	640	Petition to revive - unintentional	
142	1,280	242	640	Utility issue fee (or reissue)	
143	460	243	230	Design issue fee	
144	620	244	310	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR 1.17(q)	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	740	246	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	740	249	370	For each additional invention to be examined (37 CFR § 1.129(b))	
179	740	279	370	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)

SUBMITTED BY

Name (Print/Type)	Margaret Mary Kozik Richardson	Registration No (Attorney/Agent)	47,023	Complete (if applicable) Telephone	732-235-9350
Signature	/Margaret Mary Kozik Richardson/			Date	11/01/2002

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	:	None assigned	**Docket No.**	:	RWJ-02-79
Examiner	:	None assigned	**Dated**	:	November 1, 2002
Serial No.	:	Unknown			
Filed	:	Herewith			
Inventors	:	Ira B. Black, et al.			
Title	:	**"Genes Associated with Memory"**			

Assistant Commissioner of Patents
Washington, DC 20231
BOX PROVISIONAL PATENT APPLICATION

Sir:

Certificate of Express Mailing Under 37 CFR 1.10

For
Provisional patent application consisting of:
Specification (23 pages);
Claims (2 pages);
Sequences (3 sheets)
Drawings (3 sheets)
Provisional Patent Application Cover Sheet (1 page);
Certificate of Mailing by Express Mail;
Fee Transmittal Sheet (in Duplicate); and
Postcard

I hereby certify that the accompanying correspondence is being deposited with the "Express Mail Post Office to Addressee" service of the United States Postal Service in an envelope addressed to Assistant Commissioner for Patents, Patent and Trademark Office, Box PROVISIONAL PATENT APPLICATION, Washington, D.C. on the date indicated below. The number of the "Express Mail" mailing label **ET899942477US** has been placed on the accompanying correspondence prior to mailing. It is therefore respectfully requested that this correspondence be considered as having been filed in the Office on the date shown below in accordance with the provisions of 37 C.F.R. §1.10.

Name of Applicant, Assignee, Applicant's Attorney or Registered Representative:

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November 1, 2002
Date

Express Mail Label
No. ET899942477US

PATENT
Docket No. NJMS-02-79

Genes Associated with Memory

The present invention was supported with federal dollars and the United States Government may have certain rights in the invention.

Background

Brain-Derived neurotrophic factor (BDNF) modulates synaptic strength in hippocampal neurons, in addition to promoting survival and differentiation. To identify genes involved in trophic regulation of synaptic plasticity, we have used a multidisciplinary approach of differential display and family-specific slot blots in combination with whole-cell-patch-clamp recordings of dissociated hippocampal neurons. Three hour exposure to BDNF elicited a 2.6-fold increase in synaptic charge and a concomitant induction of 11 genes as revealed by differential display. Slot blot analysis on a population of neurons confirmed an average of 3.1-fold induction of these clones. In contrast, individual pyramidal-like neurons that were first characterized electro-physiologically in the presence of BDNF and subjected to transcriptional analysis displayed more robust increases (4.8-fold), emphasizing the neuronal heterogeneity.

Evidence now indicates that neurotrophins exert a termporal contrinuum of synaptic actions, from acute regulation of transmission to longer-term effects on synapse develpment[1,2,3,4]. In the hippocampus, which is associated with learning and memory, brain-derived neurotrophic factor (BDNF) enhances the long-term potentiation (LTP)[5,6,7] and subsequently increase synapse number[1,8]. We have found that BDNF rapidly

increases synaptic responses in dissociated hippocampal neurons[9]. However, underlying molecular mechanisms remain to be elucidated. There is evidence that both presynaptic and postsynaptic processes participate[10,9,11,6,12,15,13,14]. In the postsynaptic neuron, for example, BDNF increases synaptic strength, at least in part, via post-translational modification of NMDA receptor subunits[9,11,14,16]. Neurotrophin-induced synaptic plasticity results from such postsynaptic modifications, in conjunction with complex presynaptic mechanisms[5,6,12,13,17], the molecular basis of which is as yet unknown.

Binding of BDNF to the trkB receptor activates second messenger signaling cascades, resulting in both cytoplasmic and nuclear changes[18,19]. Acute post-translational modulation may constitute an early stage, followed by more stable changes involving altered gene expression and protein synthesis[18,20,21]. Indeed, prolonged treatment with neurotrophins promotes the development and maturation of synaptic sites[1,8,22], probably involving regulation of vesicle proteins[22,23,24]. Moreover, mice with a targeted deletion of BDNF gene exhibit impaired synaptic plasticity and depressed levels of sever vesicle proteins. Exogenous BDNF restores both physiological responses and protein levels[7,5,24]. Thus, to date, studies of BDNF-induced gene expression have focused on candidate molecules; however the full spectrum of BDNF-regulated genes and their roles in synaptic plasticity have yet to be elucidated.

Here we have combined whole-cell-patch-clamp recordings in conjunction with differential display and single-cell transcriptional analysis to identify altered expression of novel genes.

Detailed Description of the Invention

We have used differential gene profiling to begin identifying molecules that may be involved in synaptic plasticity. Our ultimate goal being to identify and define mechanisms mediating acute versus long-term activation, presynaptic and postsynaptic mechanisms, and basal versus enhanced transmission and to characterize processes underlying memory.

We have begun to identify and determine the function and utility of a number of novel sequences. Those sequences are identified as **Sequence 1, Sequence 2, and Sequence 3.**

Sequence 1 has been identified as VGF.

Sequence 2 has been identified as EFR-1.

Sequence 3 has been identified c-fos.

Each sequence is novel and contributes to learning, memory and synaptic plasticity.

Glossary

Therapeutically Effective Dose: Refers to the dose that produces the effects for which it is administered.

Patient: a mammal, preferably a human, in need of treatment of a condition, disorder or disease.

Treat and Treatment: Refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological condition, disorder or disease or obtain beneficial or desired

clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of extent of condition, disorder or disease; stabilized (i.e. not worsening) state of condition, disorder or disease; delay or slowing of condition, disorder, or disease progression; amelioration of the condition, disorder or disease state, remission (whether partial or total), whether detectable or undetectable; or enhancement or improvement of condition, disorder or disease. Treatment includes eliciting a cellular response that is clinically significant, without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

Mammal: Refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports and pet companion animals such as a household pet and other domesticated animal such as, but not limited to, cattle, sheep, ferrets, swine, horses, poultry, rabbits, goats, dogs, cats, and the like. Preferred companion animals are dogs and cats. Preferably, the mammal is human

Antagonist: includes but is not limited to, any suitable molecule, compound, protein or fragment thereof, nucleic acid, formulation or substance that can regulate activity in such a way that synaptic growth is increased. The inhibitor can include, but is not limited to the specifically identified BDNF.

Formulations and Methods of Administration

A pharmaceutical composition useful in the present invention comprises an antagonist (such as described above) and a pharmaceutically acceptable carrier, excipient,

diluent and/or salt. Pharmaceutically acceptable carrier, diluent, excipient and/or salt means that the carrier, diluent, excipient and/or salt must be compatible with the other ingredients of the formulation, does not adversely affect the therapeutic benefit of the antagonist, and is not deleterious to the recipient thereof.

Administration of the compounds or pharmaceutical compositions thereof for practicing the present invention can be by any method that delivers the compounds systemically. These methods include oral routes, parenteral routes, intraduodenal routes, etc.

For topical applications, the compound or pharmaceutical composition thereof can be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax, sugars such as lactose and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Depending on the particular condition, disorder or disease to be treated, additional therapeutic agents can be administered together with the antagonist. Those additional agents can be administered sequentially in any order, as part of a multiple dosage

regimen, from the antagonist-containing composition (consecutive or intermittent administration). Alternatively, those agents can be part of a single dosage form, mixed together with the antagonist in a single composition (simultaneous or concurrent administration).

For oral administration, a pharmaceutical composition useful in the invention can take the form of solutions, suspensions, tablets, pills, capsules, powders, granules, semisolids, sustained release formulations, elixirs, aerosols, and the like. Tablets containing various excipients such as sodium citrate, calcium carbonate and calcium phosphate are employed along with various disintegrants such as starch, preferably potato or tapioca starch, and certain complex silicates, together with binding agents such as polyvinylpyrrolidone, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tabletting purposes. Solid compositions of a similar type are also employed as fillers in soft and hard-filled gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the compounds of this invention can be combined with various sweetening agents, flavoring agents, coloring agents, emulsifying agents and/or suspending agents, as well as such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof.

The choice of formulation depends on various factors such as the mode of drug administration (e.g., for oral administration, formulations in the form of tablets, pills or capsules are preferred) and the bioavailability of the drug substance. Recently,

pharmaceutical formulations have been developed especially for drugs that show poor bioavailability based upon the principle that bioavailability can be increased by increasing the surface area i.e., decreasing particle size. For example, U.S. Patent No. 4,107,288 describes a pharmaceutical formulation having particles in the size range from 10 to 1,000 nm in which the active material is supported on a crosslinked matrix of macromolecules. U.S. Patent No. 5,145,684 describes the production of a pharmaceutical formulation in which the drug substance is pulverized to nanoparticles (average particle size of 400 nm) in the presence of a surface modifier and then dispersed in a liquid medium to give a pharmaceutical formulation that exhibits remarkably high bioavailability.

The term "parenteral" as used herein refers to modes of administration, which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous, intramedullary and intraarticular injection and infusion. A pharmaceutical composition for parenteral injection can comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal injection purposes. In this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well-known to those skilled in the art. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils

(such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The pharmaceutical compositions useful in the present invention can also contain adjuvants such as, but not limited to, preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms can be ensured by the inclusion of various antibacterial and antifungal agents, such as for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It can also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of the drugs, it is desirable to slow the absorption from subcutaneous or intramuscular injection. This can be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, can depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide, polyglycolide, and polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the

particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissues.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Administration by slow infusion is particularly useful when intrathecal or epidural routes are employed. A number of implantable or body-mountable pumps useful in delivering compound at a regulated rate are known in the art. See, e.g., U.S. Patent No. 4,619,652.

Suspensions, in addition to the active compounds, can contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures thereof.

For purposes of transdermal (e.g., topical) administration, dilute sterile, aqueous or partially aqueous solutions (usually in about 0.1% to 5% concentration), otherwise similar to the above parenteral solutions, are prepared.

The pharmaceutical compositions useful in the invention can also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and can be prepared as solutions in

saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

In nonpressurized powder compositions, the active ingredients in finely divided form can be used in admixture with a larger-sized pharmaceutically acceptable inert carrier comprising particles having a size, for example, of up to 100 μm in diameter. Suitable inert carriers include sugars such as lactose. Desirably, at least 95% by weight of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 μm.

Alternatively, the composition can be pressurized and contain a compressed gas, such as, e.g., nitrogen, carbon dioxide or a liquefied gas propellant. The liquefied propellant medium and indeed the total composition are preferably such that the active ingredients do not dissolve therein to any substantial extent. The pressurized composition can also contain a surface active agent. The surface active agent can be a liquid or solid non-ionic surface active agent or can be a solid anionic surface active agent. It is preferred to use the solid anionic surface active agent in the form of a sodium salt.

Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of the invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at room temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the drugs.

The compositions useful in the present invention can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to the compounds of the invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art (see e.g., Prescott, E., Meth. Cell Biol. 14:33 (1976)).

Other pharmaceutically acceptable carrier includes, but is not limited to, a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type, including but not limited to ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

Solid pharmaceutical excipients include, but are not limited to, starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium

stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk and the like. Liquid and semisolid excipients can be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal, vegetable or synthetic origin, e.g., peanut oil, soybean oil, mineral oil, sesame oil, etc. Preferred liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols.

Methods of preparing various pharmaceutical compositions with a certain amount of active ingredient are known, or will be apparent in light of this disclosure, to those skilled in this art. Other suitable pharmaceutical excipients and their formulations are described in Remington's Pharmaceutical Sciences, edited by E. W. Martin, Mack Publishing Company, 19th ed. (1995).

Pharmaceutical compositions useful in the present invention can contain 0.1%-95% of the compound(s) of this invention, preferably 1%-70%. In any event, the composition or formulation to be administered will contain a quantity of a compound(s) according to this invention in an amount effective to treat the condition, disorder or disease of the subject being treated.

One of ordinary skill in the art will appreciate that pharmaceutically effective amounts of the antagonist can be determined empirically and can be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug form. The agents can be administered to a patient as pharmaceutical compositions in combination with one or more pharmaceutically acceptable excipients. It will be understood that, when administered to, for example, a human patient, the total daily

usage of the agents or composition of the present invention will be decided within the scope of sound medical judgement by the attending physician. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors: the type and degree of the cellular response to be achieved; activity of the specific agent or composition employed; the specific agents or composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the agent; the duration of the treatment; drugs used in combination or coincidental with the specific agent; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the agents at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosages until the desired effect is achieved.

For example, satisfactory results are obtained by oral administration of the compounds at dosages on the order of from 0.05 to 500 mg/kg/day, preferably 0.1 to 100 mg/kg/day, more preferably 1 to 50 mg/kg/day, administered once or, in divided doses, 2 to 4 times per day. On administration parenterally, for example, by i.v. bolus, drip or infusion, dosages on the order of from 0.01 to 1000 mg/kg/day, preferably 0.05 to 500 mg/kg/day, and more preferably 0.1 to 100 mg/kg/day, can be used. Suitable daily dosages for patients are thus on the order of from 2.5 to 500 mg p.o., preferably 5 to 250 mg p.o., more preferably 5 to 100 mg p.o., or on the order of from 0.5 to 250 mg i.v., preferably 2.5 to 125 mg i.v. and more preferably 2.5 to 50 mg i.v.

Dosaging can also be arranged in a patient specific manner to provide a predetermined concentration of the agents in the blood, as determined by techniques accepted and routine in the art (HPLC is preferred). Thus patient dosaging can be adjusted to achieve regular on-going blood levels, as measured by HPLC, on the order of from 50 to 5000 ng/ml, preferably 100 to 2500 ng/ml.

Therapeutic Uses

The use of sequences as targets for high-throughput screening and as drug targets for promotion of synaptic growth for treatment associated with illness, trauma, or a genetic condition.

Examples:

The examples are provided as illustrations and not intended to limit the invention.

Example 1: BDNF regulates gene expression at 3 hr

To elucidate molecular mechanisms underlying BDNF-induced synaptic plasticity, we used embryonic rat hippocampal neurons maintained in fully defined, serum-free medium[9]. To allow sufficient time for gene induction, we chose a 3 hr BDNF exposure and then recorded synaptic responses under whole-cell voltage-clamp conditions of treated cells compared with control, untreated cells. Cells treated with BDNF (50ng/ml) for 3 hr exhibited a 2.6-fold increase in synaptic charge ($n=8$; *t* test, $p< 0.05$), see **Figures 1 A, B and D**, although there was characteristic variability among individual neurons, see **Figure 1C**. These date indicate that a 3 hr treatment is comparable with acute treatments (10 minutes) with BDNF in terms of synaptic response[9, 25]. The longer-term BDNF treatment, however, permits for transcription of immediate early genes as well as downstream genes that potentially play roles in synaptic plasticity.

Example 2: Gene Expression

Differentially expressed genes potentially involved in BDNF regulation of synaptic activity were identified by comparing patterns of mRNA expression in control cultures with those treated with BDNF or NGF for 3 hr. **Figure 2** is a representative gel selected from over 20 different gels showing amplified subsets of mRNA by use of a

combination of anchored and arbitrary primers. Amplifed cDNAs in each experimental group are similar, indicating that the general gene expression profile remains unaltered. However, selective differences among duplicates indicate induced expression of specific cDNAs after neurotrophin treatment, see **Figure 2.** Some genes appear to be induced by both NGF and BDNF, suggesting a general responsiveness to neurotrophins. We focused on genes regulated exclusively by BDNF, because NGF has not been implicated in synaptic plasticity[9, 26].

The identity of the differentially expressed genes induced exclusively by BDNF was derived from multiple gels and was determined by recovering cDNA products and reamplifying using corresponding primer sets. Partial sequence analysis and homology searches revealed that 4 of the 11 induced sequences corresponding to known genes with known functions, six cDNAs corresponded to known expressed sequence tags (ESTs) and one of the cDNAs corresponded to a novel gene not previously cloned, **see Table 1.**

Example 3: BDNF and Protein Expression

To determine whether altered gene transcription is accompanied by protein translation in this system, Western blot analysis was performed. Our initial observation of Rab3A and GC expression after a 3 hr BDNF treatment showed no obvious change in protein levels. To allow additional time for protein translation, we used a 6 hr BDNF exposure. Under these conditions, Rab3A protein levels increased 2.0±0.5-fold ($n = 4$), and GC increased 1.4±0.1-fold ($n = 3$), normalized to actin and compared with control, vehicle-treated samples, see **Figures 5 A and B.**

A number of molecules are known to regulate Rab3A activity in synaptic vesicle trafficking[27]. We analyzed their protein expression at 6 hr to evaluate potential involvement of BDNF-induced synaptic plasticity. Two proteins that regulate Rab 3A activity, Rab GAP and Rab GDI, showed enhanced expression after BDNF application, whereas Rab GEP was not altered, see **Figures 5 A and B.** IN addition, Rabphillin and RIM, two proteins that interact with Rab 3A to facilitate the anchoring of synaptic vesicles to the plasma membrane[28, 29], were also upregulated by BDNF, see **Figures 5 A and B.** Minimal transcriptional changes were observed for these regulatory proteins by reverse transcription-PCR. Taken together, these data indicate that transcriptional changes of Rab 3A by BDNF are also accompanied by translational regulation. Furthermore, as we have shown above for the GC pathway, molecules associated with the Rab 3A pathway can be regulated by BDNF treatment and therefore are involved with neurotrophin induced synaptic plasticity.

Figures

Figure 1: Three hour BDNF treatment potentiates synaptic activity in dissociated hippocampal neurons. *A, B* Example whole-cell voltage-clamp recordings (Vhold = -60 mV) from one control hippocampal neuron (*A*) and one neuron exposed to BDNF (20 ng/ml) for 3 hr (*B*). *C*, Effect of BDNF on synaptic charge in population of neurons. Each triangle represents the average synaptic charge for one neuron during a stable 10 minute recording period. *D*, Average of the individual cells shown in *C*. BDNF

increased synaptic charge 2.6-fold compared with control cells (n=8; *p<0.05). Recordings were obtained from multiple platings.

Figure 2: A number of genes are differentially expressed in BDNF-treated cultures. Total RNA from control (lanes 1, 2), NGF-treated (3 hr; 50 ng/ml; lanes 3, 4), and BDNF-treated (3 hr; 50 ng/ml; lanes 5,6) sister cultures were subjected to differential mRNA analysis. A representative autoradiogram of amplified PCR products is shown for one set of primer pairs that identified distinct fragments (⇐) with differential expression in duplicate neurotrophin-treated groups.

Figure 5: BDNF induces translation of identified genes and their regulatory proteins in hippocampal neurons. *A*, equal amounts (50 μg) of protein from control and BDNF-treated (6 hr; 50 ng/ml) cultures were loaded in each lane, electrophoreses, immunoblotted with antibodies, and visualized with ECL. GC (70 kDa) and Rab 3A (25 kDa) are upregulated by BDNF treatment. Several regulatory proteins were also induced by BDNF: RIM (173 kDa), Rab GAP (120 kDa), Raphilin (75 kDa), and Rab GDI (50 kDa). Rab GEP (210 kDa) was unaltered. Actin (42 kDa) levels remain unchanged. *B*, Quantitation of increase in protein levels in BDNF-treated samples relative to control cells after normalization to actin is shown (n = 4, except for GC, n = 3 and Rab GEP, n = 2).

Table 1: DNA from putative differentially expressed bands found in duplicated samples was reamplified by PCR using the appropriate primer combination. CDNA fragments were subcloned, and five separate clones from each fragment were sequenced. Comparisons of unknown nucleotide sequences to known sequences in the GenBank and EMBL data bases were performed using the BLAST program. The cDNAs ranged from 251 to 801 bp, and all of them were upregulated by BDNF.

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signaling in BDNF-induced synaptic potentiation in cultured hippocampal neurons. Proc Natl Acad Sci USA 95: 10884-10889.

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Claims

We claim the following

1. The novel sequence identified as sequence 1.

2. The novel sequence identified as sequence 2.

3. The novel sequence identified as sequence 3.

4. The use of the sequence as in Claim 1 for the purpose of high through put screening

5. The use of the sequence as in Claim 2 for the purpose of high through put screening.

6. The use of the sequence as in Claim 3 for the purpose of high through put screening.

7. The antagonism of sequence 1 to effect a response that would result in increased synaptic growth.

8. The antagonism of sequence 2 to effect a response that would result in increased synaptic growth.

9. The antagonism of sequence 3 to effect a response that would result in increased synaptic growth.

10. The treatment of an illness, trauma or genetic condition associated with damaged or diseased synapses using the method as described in Claim 7.

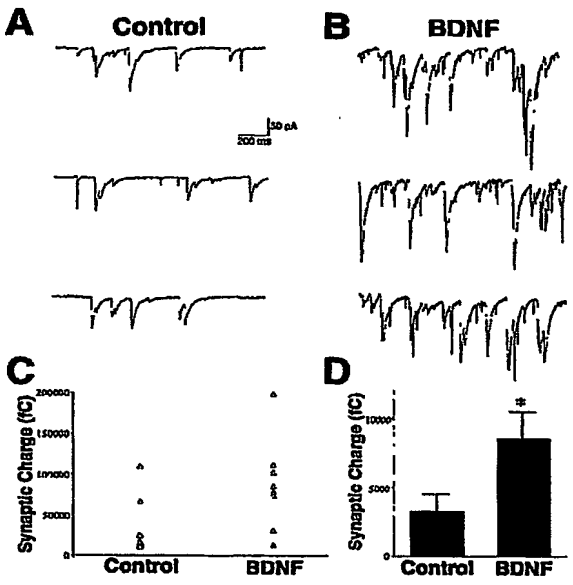
11. The treatment of an illness, trauma or genetic condition associated with damaged or diseased synapses using the method as described in Claim 8.

12. The treatment of an illness, trauma or genetic condition associated with damaged or diseased synapses using the method as described in Claim 9.

13. The treatment of Alzheimer's Disease using the method as described in Claim 7.

14. The treatment of Alzheimer's Disease using the method as described in Claim 8.

15. The treatment of Alzheimer's Disease using the method as described in Claim 9.


A
Control
B
BDNF
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200 ms
C
Synaptic Charge (fC)
Control
BDNF
D
Synaptic Charge (fC)
*
Control
BDNF

Figure 1

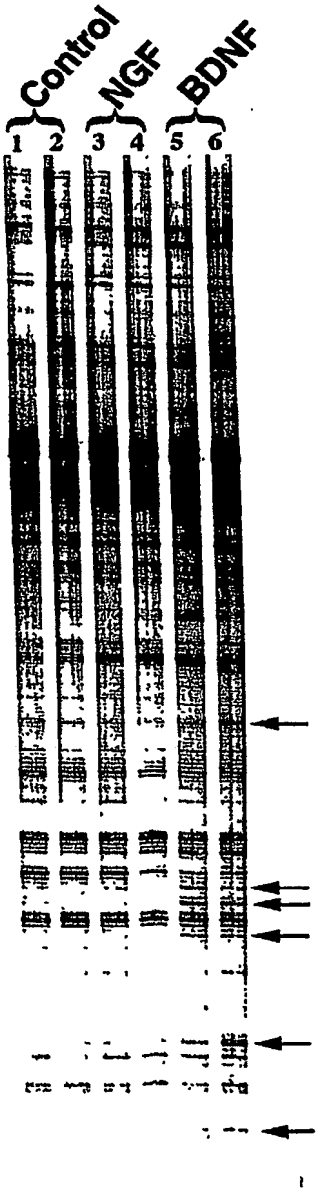
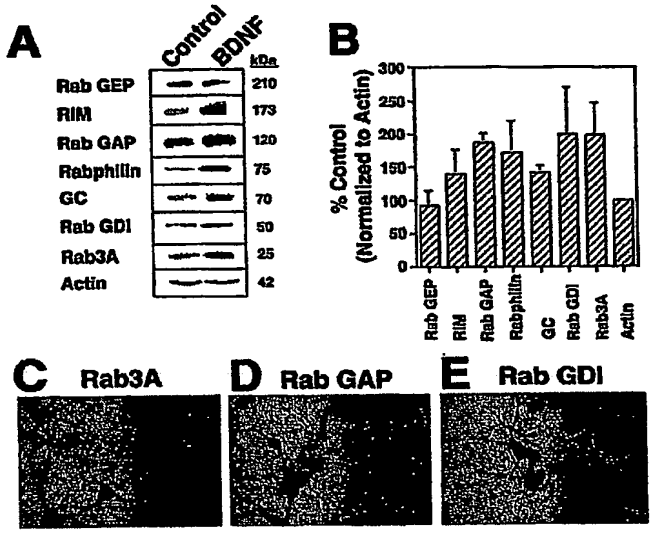


Figure 2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Control
BDNF
kDa
Rab GEP
RIM
Rab GAP
Rabphilin
GC
Rab GDI
Rab3A
Actin
210
173
120
75
70
50
25
42
B
% Control
(Normalized to Actin)
300
250
200
150
100
50
0
Rab GEP
RIM
Rab GAP
Rabphilin
GC
Rab GDI
Rab3A
Actin
C Rab3A
D Rab GAP
E Rab GDI

Figure 5

VGF: Sequence 1

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1621 cttagtcttc tcatagcatt aactaatctg ttgggttcat tattggaatt aacctggtgc
1681 tggatatttt tcggattgta tctagtgcag ctgattttaa caatacctac tgtgttcctg
1741 gcaatagtgt gttccaattt agaaatgacc aatattaaac taagaaaaga tagaacttta
1801 ttttccggta gatagaaata aatcgctata tccacgtact gtagctcttc agcgtccatg
1861 ttcattgtca tgtaactgat catgcattgt tgaggtggtc tgaatgttct gacattaaca
1921 gttttccatg aaaacgtttt attgtgtttt caatttattt attaagatgg attctcagat
1981 atttatattt ttatttttatt tttttctatc ctgaggtctt tcgacatgtg gaaagtgaat
2041 ttgaatgaaa aaattttaag cattgtttgc ttattgttcc aagacattgt caataaaagc
2101 atttaagttg aatgcg
```

Table 1. Analysis of cDNA fragments identified by mRNA differential display

Clone number	Fragment size	Regulation	Sequence homology	Accession number
C1e	514 bp	Up	ypt1/Rab3A	X15747
C6d	325 bp	Up	EST	AA891880
A1b	584 bp	Up	EST	AA849956
A1c	584 bp	Up	EST	AI137233
A3a	350 bp	Up	Guanylate cyclase A	JO5677
A3c	356 bp	Up	Unknown	U25489
A5d	801 bp	Up	EST	AA997878
A6c	259 bp	Up	ATP synthase	AF115771.1
G1b	251 bp	Up	Estrogen-induced gene	S74324
G2a	578 bp	Up	EST	C81615
G2c	628 bp	Up	EST	AI117373

Table 1